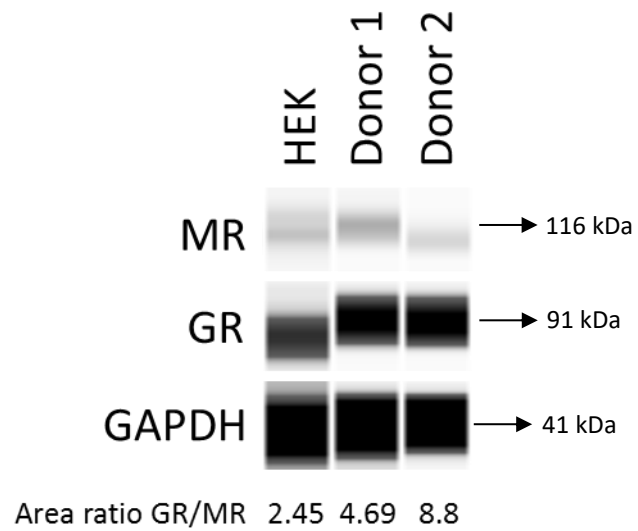


Supplementary Figure 4. An overview of the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) expression on the mRNA and protein level. **(A)** Gene expression of the MR and the GR relative to the housekeeping gene *LRP10* in donors that were treated with either aldosterone or spironolactone. For 3 donors, we have performed Magnetic Activated Cell Sorting (MACS) and isolated the RNA immediately after dissecting and digesting the choroidal tissue, to rule out for the possibility that MR expression had diminished after cell culture. **(B)** Protein quantification of the GR, MR, and GAPDH as housekeeping protein. Wes automated Western blotting and analysis was performed according to manufacturer's instructions (ProteinSimple, San Jose, California). Samples were diluted to adjust protein concentration to 0.8 µg in 4 µl with sample buffer. The antibody for both the GR (ThermoFisher Scientific, Waltham, MA, USA, Cat. No. MA1-510) and the MR (rMR1-18 1D5, a kind gift from Dr. C. E. Gomez-Sanchez, University of Mississippi Medical Center) were diluted 1:10, and the GAPDH antibody (Santa Cruz Biotechnology, Dallas, Texas, Cat No. sc-25778) was diluted 1:100. Choroidal endothelial cells of 2 different donors were purified with 2 MACS procedures according to our described protocol and lysed in radioimmunoprecipitation assay (RIPA) buffer. Human embryonic kidney cells, also lysed in RIPA buffer, were included as a reference.

Abbreviations: ΔCt = difference in cycling threshold value relative to the housekeeping gene, kDa = kilodalton, HEK = human embryonic kidney cells.

| Figure | Aldosteron treatment | Spirolacton treatment | Culture / Direct MACS isolation | MR expression (average ΔCt) | GR expression (average ΔCt) |
|--------|----------------------|-----------------------|---------------------------------|--------------------------------------|--------------------------------------|
| S3A | Yes | No | Culture | 3.4 | 1.8 |
| S3B | Yes | Yes | Culture | 8.0 | 1.6 |
| S3C | Yes | Yes | Culture | 2.2 | 2.2 |
| S3D | No | Yes | Culture | 4.0 | 1.7 |
| S3E | Yes | Yes | Culture | 6.3 | 2.2 |
| | - | - | Direct MACS | 2.9 | 1.4 |
| | - | - | Direct MACS | 1.3 | 0 |
| | - | - | Direct MACS | 2.9 | 1.7 |

A



B